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Use of Surfactants in the Bioremediation of Petroleum-Contaminated Soils

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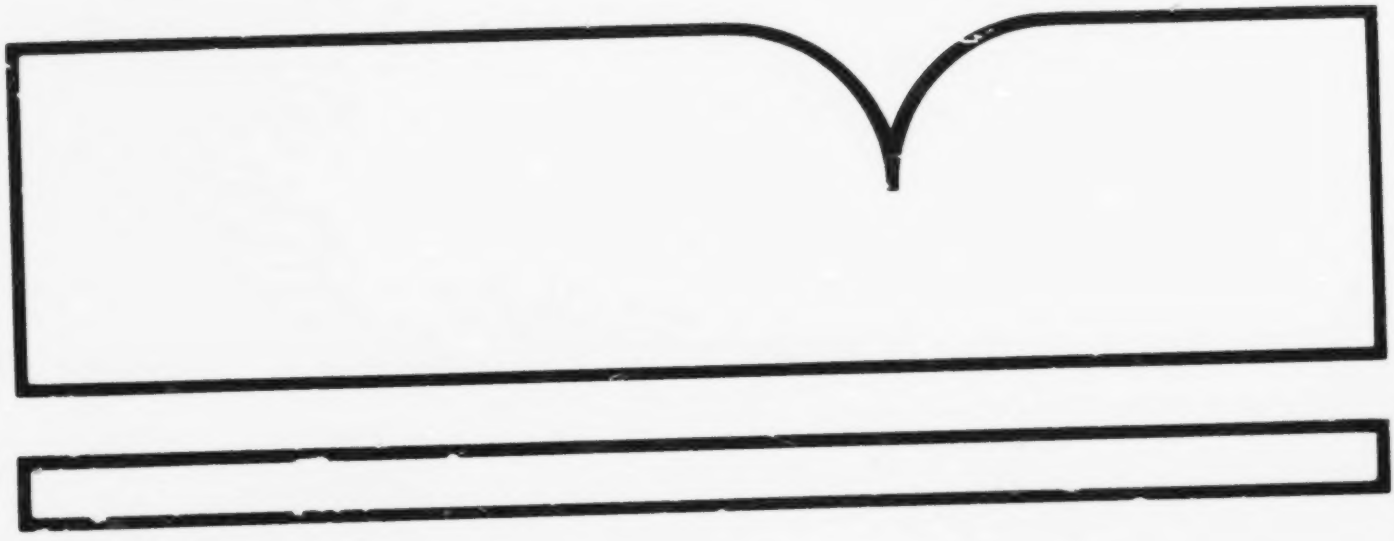

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THE USE OF SURFACTANTS IN THE BIOREMEDIATION OF PETROLEUM-CONTAMINATED SOILS

by Guy Green

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THE USE OF SURFACTANTS

IN THE BIOREMEDIATION OF PETROLEUM CONTAMINATED SOILS

Summary:

Surfactants were considered as a possible method of increasing the rate of bioremediation of soils and groundwater contaminated with petroleum hydrocarbons. A large number of surfactants have been demonstrated to be biodegradable under aerobic conditions. Soil, petroleum hydrocarbon and surfactant interactions dictate that for a given set of conditions, only certain surfactants will significantly lower the surface and interfacial tensions and thus be effective. Emulsifiers have been found to increase the rate of bioremediation but have not been found to increase the extent of biodegradation to which a petroleum product will undergo. Most petroleum products are made up of a complex mixture of hydrocarbons. Different hydrocarbon compounds have been found to be biodegraded at differing rates. The rate and extent to which a particular constituent will degrade is based on its classification and physical properties.

INTRODUCTION

Leaking underground storage tanks (LUST) have become an ever developing problem in the United States in the last several years. Contaminated soils and groundwater have caused many cited cases of health and safety hazards. The purpose of this paper is to address one of the methods of remediating contaminated soil and groundwater, enhanced bioremediation and the use of emulsifiers to increase the effectiveness of this method.

BACKGROUND OF THE PROBLEM

Underground storage tanks experience wide use across the United States today. They may be found at airports, service stations, farms, schools, as well as industrial and manufacturing complexes. Over 90% of these tanks store petroleum products. National estimates indicate that up to 35% of these tanks are leaking their products into the environment. [WA Toxic Sub. Fact Sheet]

Many of the spills occur from small, pinhole leaks that slowly release their product into the surrounding soil for long periods of time. Substantial amounts of petroleum hydrocarbon can be leaked into the environment before being detected. Contamination associated with underground storage tanks can also occur from sudden, large releases.

When a leak occurs, petroleum hydrocarbon is transported through the soil principally by three mechanisms; mass flow of product, liquid diffusion within the soil solution, and diffusion of the vapor within the soil air voids [Hern and Melancon]. A petroleum product, such as gasoline, is actually made up of a complex mixture of components. Each of these specific chemicals has its own

specific properties [Nyer and Skladany]. Vapor diffusion will be most affected by the vapor pressures of the compounds involved. The greater the vapor pressure, the greater the driving force for the component to enter the gaseous phase and diffuse away from the original site of the spill. Liquid diffusion is the transport of dissolved solutes within the soil water via intermolecular collisions that serve to dissipate higher concentration into lower ones. This mechanism has its greatest effect when free phase is in contact with a moving water table.

In most cases, the dominant mechanism for transport away from the original spill is mass flow of the petroleum product. This behavior will be most influenced by the permeability and the wetness of the geological materials receiving it [Wilson and Ward]. If the soil is dry, the petroleum product will be drawn into the earth by capillary action. The main body of material will be drawn down by the action of gravity. As the hydrocarbon reaches down into wetter regions of the soil, capillary attraction becomes less important. Petroleum will move by the displacement of air. If the spill is large enough, petroleum will eventually reach the water table. Most oils will float on top of the water table and spread laterally. The petroleum in the region above the water table will eventually drain, leaving behind some material known as residual saturation [Wilson and Ward].

Wilson and Brown [1989] report that for a "typical" 30,000 gallon gasoline spill, about 62% of the gasoline will remain as free phase while 33% will be adsorbed to the soil and 1-5% will be dissolved in the groundwater. The volume of soil contaminated by the gasoline will be just the opposite in relative volume. The free phase will occupy about 1%, the adsorbed phase about 20%, and the dissolved phase as much as 79%. A treatment process must be able to

remediate all three phases to be effective.

One of the techniques that has proven successful in restoring a contaminated subsurface environment such as the one described above is bioremediation. In situ bioremediation is the use of naturally occurring microorganisms to treat the petroleum product that has infiltrated the subsurface environment [Wilson and Sims, 1989]. Ward and Lee [1988] state that successful application of in situ restoration depends upon the presence of metabolically appropriate microflora and a subsurface environment that is readily permeable. In addition, substances that are inhibitory to microbial action on the hydrocarbon can not be present [Lee et. al.,1988].

Soil microorganisms that can utilize the petroleum hydrocarbon as their substrate will begin to grow and degrade the contaminant. As they do so, they will soon deplete oxygen and other nutrients necessary for their biological activity. The major goal for in situ remediation is to supply the oxygen and the necessary nutrients for continued biological action upon the petroleum hydrocarbon [Fogel and Findlay]. The task of the engineer is to supply the necessary ingredients in as efficient a method as possible so that biodegradation of the contaminant can be carried out to its fullest extent.

OBJECTIVES

The objective of this report is to explore the use of emulsifiers to enhance the bioremediation of petroleum products in contaminated soils and ground water. The process of bioremediation has been shown to be an effective method of removing contaminants from the subsurface environment. The use of emulsifiers to increase the effectiveness of the bioremediation process will

be explored. This paper will address six major aspects. These are:

1) a list of the biodegradation potential of surfactants, as well as the effects of surfactants on human health and the viability of microorganisms.

2) the behavior of surfactants in various soil types and conditions;

3) the effects of emulsifier addition on nutrient and oxygen uptake by the microorganisms;

4) quantification of the increase of solubilities of petroleum product constituents with emulsifier use;

5) comparison of the human health risks from non-emulsified groundwater in contact with petroleum-contaminated soils with emulsifier-added groundwater undergoing biological degradation;

6) existence of unsafe degradation daughter products which could accumulate in groundwater due to recalcitrance to biodegradation.

SURFACTANT BIODEGRADATION

Use of surfactants first became widespread during the 1950's when they were found to be both effective and economical. With the widespread use of these products their effect upon the environment were soon noticed. Secondary wastewater treatment plants were not effectively removing these contaminants from the wastewater before its release to the environment. Research revealed that the main product resistant to biodegradation was alkylbenzenesulfonate (ABS) made from tetrapropylene (TBS) [Swisher, 1970]. Several products were then developed that would be less persistent in the environment. One of the main products developed at that time was linear alkylbenzenesulfonate (LAS). It was found to biodegrade to environmentally acceptable levels.

Swisher has done extensive work on the biodegradation of surfactants. He

reports that the soil is a powerful agent for degrading organic compounds. Soil systems were found to be one of the most effective agents for biodegradation. In order for the soil microorganisms to be effective in degrading surfactant compounds aerobic conditions must be maintained. Under anaerobic conditions surfactant compounds are much more persistent in the subsurface environment. In Swisher's testing aerobic conditions were achieved by maintaining unsaturated conditions by intermittent feeding so that air was able to diffuse into the pore spaces. When saturated conditions dominated, the oxygen was quickly utilized and biodegradation essentially ceased.

Soil lysimeters were found to have 98% primary biodegradation of TBS, as well as high ultimate biodegradation as revealed by the release of inorganic sulfates [Swisher 1987]. TBS has been found in various other tests to be the slowest of surfactants to biodegrade. These results would indicate that other surfactants, developed for their higher biodegradation properties, would also be biodegradable in the soil.

Swisher, in his book Surfactant Biodegradation, lists extensive tables on specific surfactants and their biodegradation potential. The six categories that he lists are 1) chemical nature of the substrate, 2) the extent of degradation, 3) the general biological conditions, 4) the exposure time, 5) the analytical method, and 6) the reference. The information in these tables could prove to be useful to a researcher in that once a surfactant is selected for possible use in an underground situation the relative biodegradation of that compound can be determined.

In a 1979 report by Texas Research Institute, Inc. (TRI), they listed the biological and physiological characteristics of the surfactants they tested for displacement properties. The toxicity data as listed in the TRI report is

listed in Table 1.

TABLE 1

BIOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS
Texas Research Institute, 1979

Nonisol 210	Low level of toxicity to animals. Ester linkage hydrolyzed by pancreatic lipase to fatty acid and polyetheylene glycol which is excreted in urine and feces.
Alrosol 0	No information
Amine 0	Acute oral LD50 Rats - 800-900 mg/kg; causes skin burns and eye injury to rabbits.
Sarkosyl L	Acute Oral LD50, Rats 3000 mg/kg; not irritating to skin or eyes of rabbits; inhibits pancreatic amylase and lipase.
Lodyne flouro-	Practically nontoxic, moderately to surfactants minimally irritating to eyes and skin.
Aerosol OT	Acute Oral LD50, Mice - 1000 mg/kg; eye irritant; non-irritating to skin.
Aerosol MA	Acute oral LD50, Rats - 1750 mg/kg; skin adsorption LD50, Rabbits 5.0 ml/kg; severe irritant to eye and skin of rabbit
Aerosol 18	Acute oral LD50, Rats - 2680 mg/kg (35% A.I.); not irritating to rabbit skin; moderate irritant to eye.
Aerosol C-61	Acute oral LD50, Rats - 2850 mg/kg (70% A.I.); Severe irritant to eye and skin of rabbit.
Alfonic 1412-S	Biodegradable; not considered hazardous; avoid contact with eye and skin.
C-550 Slurry	Moderate skin irritant, can cause eye irritation; considered non-hazardous.
Base 11-T	No information
Surfatron DT-41	No information

Richonate YLA	Generally considered non-hazardous, generic FDA approval for food contact uses.
Sol-Fon-Ate AA-10	Non-biodegradable
Zonyl A	Biodegradable with acclimated bacteria; low order of toxicity (rats); not irritating to skin; mild eye irritant.
Merpal LF-H	Biodegradable
Product BCO	No information
Alkanol WXN	No information
"Duponol" WAQE	No information
"Duponol" G	No information
Zonyl FSA	Acute oral LD50, Rats - 1500 mg/kg; non-irritating to skin (Guinea pig); minimally irritating to eye (rabbit); wash hands before smoking.
Zonyl FSN	Acute oral LD50, Rats-1700 mg/kg; non-irritating to skin; minimal irritation to eye; wash hands before smoking.
Zonyl FSB	Acute oral LD50, Rats - >25,000 mg/kg; non-irritating to skin; severe eye irritant; wash hands before smoking.
Hyonic PE series	FDA approval for indirect food contact.
Poly-Tergent B series	Considered non-hazardous to health; should not be used in preparations for internal consumption or application to skin without approval of Federal or State authorities.
Surfynol TG	Acute oral LD50, Rats - 1800 mg/kg; not considered to be toxic.
Surfynnol TG-E	Oral LD50 - 5400 mg/kg; not considered to be toxic; approved as ingredient of pesticide formulations applied to food crops.
Surfatron DT-41	No information

The most extensive studies of biodegradation of oil-water-emulsion systems have been in the use of dispersants for marine oil spills. It has been shown

that some emulsions are effective in increasing the rate at which biodegradation occurs but not the extent to which it occurs [Atlas and Bartha, Mulkins-Phillips and Stewart, 1974]. Mulkins-Phillips and Stewart also found that for the four dispersants they tested, only one dispersant increased the biodegradation rate while all four caused microbial population shifts. Colwell and Walker [1977] report that some dispersants are toxic to microorganisms by lowering the surface tension below 45 dyn/cm; the optimum surface tension for oil degrading bacteria being between 45 and 60 dyn/cm. Thus they claim that some lowering of the surface tension will be advantageous, but too much may be lethal or at least inhibitory. Although the actual numbers are likely to vary for a subsurface environment, the same general principles will probably be found to apply.

SURFACTANT-SOIL BEHAVIOR

As mentioned earlier, a petroleum product is actually a complex mixture of components. Table 2, taken from Nyer and Sklandany [1989], lists some major fractions, their distillation numbers, and the range of carbon numbers within their make up.

TABLE 2
PETROLEUM DISTILLATION PRODUCTS
(Nyer and Skladany, 1989)

Fraction	Distillation Temperature, C	Carbon Number
Gas	Below 20	C-1 to C-4
Petroleum Ether	20 to 60	C-5 to C-6
Ligroin(light naptha)	60 to 100	C-6 and C-7
Natural Gasoline	40 to 205	C-5 to C-10 and cycloalkanes
Kerosine	175 to 325	C-12 to C-18 and aromatics
Gas Oil	Above 275	C-12 and higher
Lubricating Oil	Non-volatile liquids	Probably long chains attached to cyclic compounds
Asphalt	Non-volatile solids	Polycyclic structures

Each of these specific chemicals will have its own properties and subsequently its behavior in the subsurface will be different. Table 3 (also from Nyer and Skladany) lists physical and chemical properties for selected petroleum hydrocarbons.

TABLE 3
PHYSICAL/CHEMICAL PROPERTIES OF SELECTED
PETROLEUM HYDROCARBONS
(Nyer and Skladany, 1989)

Compound	Molecular Weight	Density	Solubility ppm(@C)	Boiling Point,C	Vapor Press. @1 atm &(C)
Pentane	72.5	0.626	360(16)	36	430(20)
Hexane	86.17	0.66	13(20)	68.7	120(20)
Decane	[illegible].28	0.73	0.009(20)	173	2.7(20)
Cyclopropane	42.80	0.72	37000	-33	760(-33)
Cyclopentane	70.14	0.751	<1000	---	200(13.8)
Cyclohexane	84.16	0.779	55(20)	81	77(20)
Benzene	78.11	0.878	1780(20)	80.1	76(20)
Toluene	92.1	0.867	515(20)	110.8	22(20)
ortho-Xylene	106.2	0.88	175(20)	144.4	5(20)

Benzene, for example, has a significantly higher solubility than toluene

or xylene. Benzene will therefore tend to dissolve into groundwater to a higher degree. In general, the amount of a chemical adsorbed to the soil will also be influenced by its properties. The more hydrophobic a compound, the greater its adsorption potential.

Also of significance is the properties of the soil receiving the contaminant. The adsorption properties of a soil serve to retain a certain amount of hydrocarbon as the petroleum passes through the soil column. Jury [1986] reports that a positive linear relationship lies between the soil organic matter content and adsorption of organic chemicals. Clay mineral content, because of its net negative charge, will also increase the adsorption reaction of virtually all compounds. Other factors, such as soil water content and porosity, will influence the amount adsorbed. Tuck et al. report that capillary forces are not likely to be very influential in the movement of petroleum products. Non-polar liquids are unlikely to be the wetting solution. This is especially true in the presence of silicates, which often comprise a major component of the soil make-up. Capillary suction requires a wetting solution.

In a couple of parallel studies, the Texas Research Institute (TRI) [1979, 1985] studied the effects of surfactants on underground movement of petroleum hydrocarbons. In the first study they examined the interfacial tensions of various surfactant-gasoline-deionized water systems for a surfactant system that demonstrated the most potential for further study. They determined three systems proved to be the most promising for further study. These were:

a) Richonate-YLA and Hyonic PE-90, the lowest tension anionic and nonionic,

b) Alfonic 1412-S, a normal alkyl polyether sulfate, and Alrosol O,

a fatty acid amide, and

c) Poly-Tergent B-500, a polyethoxylated nonyl phenol with an average of 15 molecules of ethylene oxide per molecule. TRI conducted further studies on Richonate-YLA, Alrosol-O, and Hyonic PE-90.

One dimensional analysis was conducted by TRI using three types of sand: a 20-50 mesh local river sand, a 50-100 mesh local river sand, and a 20-50 mesh white Ottawa sand. TRI found that Richonate-YLA was effective in displacing gasoline but that it and gasoline formed a viscous emulsion that resulted in a low flow rate. (They found this to also be true with Alrosol-O/gasoline emulsions.) It was determined that plugging could be remedied by adding Hyonic PE-90, a nonionic surfactant to the Richonate YLA solution.

A 2% Richonate YLA + 2% Hvonic PE-90 solution was added to sand packs after gasoline being removed by water no longer flowed as a separate phase from the cores. TRI found that approximately 80% of the residual gasoline was removed by the surfactant solution. At the end of the flooding (after 5 pore volumes eluted) the surfactant solution leaving the core contained 50 ppm of gasoline. After 20 pore volumes of flooding by water only, gasoline concentrations were present at 150 ppm. They further state that the Richonate-YLA/Hyonic PE-90 solutions were unstable.

In the second study by Texas Research Institute, the 2% Richonate-YLA/ 2% Hyonic PE-90 solution was tested in a 10ft x 10ft x 4ft sand-filled tank. The water table was set at one foot above the tank floor. Twenty gallons of gasoline (unleaded, 87 octane) were "spilled" in the tank in each of the three test conditions. These were: a) a single application of surfactant solution, b) multiple applications at the top of the sand bed which percolated down through the sand, and c) multiple injections via injection wells within the

sand bed.

TRI stated that two factors clouded the results of their work. These were a difference in permeability between the control (no water) and the latter two surfactant solution runs, and an inability to completely account for the mass of gasoline in the experimental runs. Never the less, they found that multiple applications via either percolation or injection were effective in lowering the final residual gasoline saturation by a factor of two or three. The percentage of gasoline removed based on the amount remaining at the beginning of the surfactant application was 83% by multiple injection, 76% by multiple percolation and only 6% by single percolation.

In the conclusions of their report TRI concludes that further testing is needed to better understand the uncertainties associated with predicting distribution and flow patterns in even a simply structured soil. They recommend testing with other promising surfactant solutions as well as increased instrumentation. Additional testing is needed so that better quantification of flow dynamics is achieved. This is certainly no less true when the use of surfactants is combined with the process of bioremediation.

The U.S. Environmental Protection Agency Releases Control Branch and the U.S. Air Force Engineering and Services Center engaged in a joint project of field testing in situ washing of a fire training pit at Volk Air National Guard Base at Camp Douglas, Wisconsin. The pits had been used for firefighting training from World War II until deactivation in 1979. The subsurface soil was determined to be contaminated primarily with a medium weight oil (2,000 - 25,000 mg oil/kg soil). Also present (600 - 3500 ppb) were chlorinated volitiles dichloromethane, chloroform, 1,1,1 trichloroethane and trichloroethylene.

Two types of surfactants were used as wash solutions, "natural" and "synthetic". Synthetic surfactants were man-made products and are commercially available while natural surfactants had their origins at the fire training pit and were the by-products of biological activity. The synthetic surfactants used were:

1. Surfactant 1 (S1). A mixture of ethoxylated fatty acids by Witco Chemical Corporation. Used in agriculture as a soil penetrant.

2. Surfactant 2 (S2). An ethoxylated alkyl phenol sold by Diamond Shamrock.

3. Surfactant 3 (S3). An anionic sulfonated alkyl ester sold by Diamond Shamrock.

The natural surfactants were described as:

1. Old natural surfactant - from the first 10,000 gallons pumped from the well field.

2. New natural surfactant - from the well field after pumping 20,000 gallons, lower in suspended solids than the old natural surfactant.

3. Clarifier effluent - lime treated well field effluent lower in iron and therefore less likely to plug the soil pores with precipitates.

These solutions were added to a series of test cells, five of which were 2ft x 2ft x 1ft deep and five of which were 1ft x 1ft x 1ft deep. Their listed wash solution volumes and concentrations are given in Table 4.

TABLE 4
WASH SOLUTION VOLUMES AND CONCENTRATIONS FOR
VOLK FIELD - SEPTEMBER 1985
EPA 600/2-87/110

Pit #	Wash Solution	Concentration (% wt)	Total Volume Gallons	Pore Volumes
1	natural surfactant + S3	0.025%	84 plugged	7
2	new natural surfactant	0.024	122	10
3	old natural surfactant	0.024	168	1[illegible]
4	new natural surfactant	0.024	112	9
5	old natural surfactant	0.024	112 plugged	9
6*	clarifier effluent	0.015	28	9
7*	S3	0.5	42	14
8*	50/50 S1/S2	1.5	42	14
9*	clarifier effluent	0.015	28	9
10			0 plugged	0

* 1ft x 1ft x 1ft cel[illegible]

In a previous laboratory study a column was packed with Volk Field soil and washed with 12 pore volumes of the 50/50 S1/S2 blend. They report that the results of these tests were highly encouraging.

The conclusion of the testing was that the use of surfactants in in situ soil washing did not measurably work. The Volk Field researchers reported there was no obvious cause for the ineffectiveness of the surfactant solutions. They propose the ineffectiveness could have been due to major amounts of by-passing of contaminated zones by the wash solutions. This was backed up by Total Organic Carbon (TOC) measurements, that in some cases varied by an order of magnitude from soil samples taken in close proximity to each other. Variance in TOC measurements occurred both before and after in situ soil washing. It should also be noted that the surfactant solution concentrations used at the Volk Field tests were below the concentration levels found to be necessary by the tests done by Texas Research Institute as cited above.

EFFECTS UPON NUTRIENTS AND OXYCEN UPTAKE

Robichaux and Myrick studied the effects of six emulsants on the oxygen uptake of microorganisms in an oil-water solution. Table 5 lists the uptake rates for the various systems.

TABLE 5

OXYGEN UPTAKE RATES FOR VARIOUS SYSTEMS

Chemical System	Uptake Rate Slope (ul/hr)	Rank
BDOWH 2	69.5	1
BDOWE 8	52.5	2
BDOWE 7	28.0	3
BDOWB 1	25.6	4
BDOWH X	8.9	5
BDOWH 10	6.7	6
Oil (only)	7.8	--

These dispersing agents were propietary formulations obtained from unidentified suppliers. All that was known was that the surfactants were anionic and nonionic chemicals in organic solvents. As can be seen in the table, the first four formulations significantly increased the oxygen uptake rate, while the later two showed little effect.

As is mentioned in other sections of this report, most surfactants have been shown to be biodegradable. In situ biological treatment relies upon the action of microbes to degrade the contaminants. From the substrate energy as well as material for the synthesis of new cell mass is derived. Any surfactant added to the subsurface environment will need to be consumed by the microorganisms for the completion of the project. This will cause an increase in cell mass and an increase in the amount of oxygen required by the microorganisms. Both of these aspects are potential problems in the

bioremediation project. An increase in cell mass results in less pore space for the flow of fluids through the soil. This will result in slower flow rates if not clogging of flow altogether. Lee et al. [1986] report that supplying oxygen to the ground water is likely to be the limiting factor in the biostimulation process, especially in low permeability aquifers. If this is indeed true then lack of oxygen and not a lack of microbial cell/contaminant contact is limiting the completion of the project. Furthermore, adding surfactant will increase the oxygen demand in that oxygen will be required by the microbes to break down the surfactant itself.

SURFACTANT - SOLUBILITY BEHAVIOR

Morgan et. al [1977] have studied interfacial tensions of various oil-water-surfactant solutions. They report that a specific hydrocarbon mixture, in interacting with a series of surfactant-aqueous solutions, will exhibit an equivalent alkane carbon number (EACN). A specific surfactant solution will give a minimum interfacial tension at a certain EACN. Thus a certain petroleum product, spilled into the environment, will exhibit an EACN. Only certain surfactants, or surfactant-cosurfactant mixtures, will significantly lower the interfacial tension. In summarizing their results, they list the effects of key variables on Nmin, the carbon number of the alkane with the lowest surface tension for the specific surfactant solution. Table 6 lists their findings.

TABLE 6
FACTORS AFFECTING SURFACTANT-INTERFACIAL
TENSION PROPERTIES
Morgan et. al

VARIABLE INCREASED	Increased	None	Decreased
Surfactant Mol. Wt.	X		
Branching of Surf. alkyl structure	X		
Conc. of pure surfactant		X	
Conc. of complex surf. mixture			X
Electrolyte concentration	X		
System temperature			X
Age of system with pure surfactant		X	
Age of system with complex surf.			X

Many commercial surfactants are actually a complex mixture of chemicals with an average molecular weight as specified by the manufacturer. In the manufacturing process no one single molecular structure is produced. Instead, the product falls into a narrow range of closely related molecules. These are what are referred to as complex surfactants.

HEALTH RISKS FROM EMULSIFIED GROUNDWATER VERSES NON-EMULSIFIED GROUNDWATER

To date on the publishing of this report, the author was able to find no data concerning persistent, harmful end products left from bioremediation projects using emulsifiers. Indeed, data are lacking on the specific compounds left from bioremediation projects as a whole. The success of bioremediation in the literature is being judged on the disappearance, or lack thereof, of the original petroleum hydrocarbon constituents. Some tests will also analyze CO_2 production and/or organic matter increases.

In the section following this one, "Biodegradation Products", the biodegradation potential of petroleum hydrocarbons is discussed according to

their classifications. It can be seen from that section that some compounds will tend to be more persistent than others. In the reactions involving pure petroleum hydrocarbons, the end products of a particular reaction tend to more closely resemble natural organic material. The most toxic compounds will tend to be those fractions of the original petroleum products that resist biodegradation.

BIODEGRADATION PRODUCTS

Biodegradation of oil products in the soil involves complex interactions between the initial oil product constituents, degradation intermediates, and the microorganisms involved. A greater extent of biodegradation can almost always be achieved with a mixed culture of microorganisms over that achieved by a pure culture. The metabolic end products of one microorganism are often utilized as substrate by another. The metabolic routes used by a microorganism to break down a specific hydrocarbon can differ from the method used by another microorganism to break down the same hydrocarbon compound. Indeed, the metabolic route used by a microbe can change during the life of a project. Some microorganisms originally transform a certain product by co-metabolism, a process defined as "... the degradation of a compound only in the presence of other organic material which serves as the primary energy source." [Brock et al.]. Later the microorganism might adapt to using the product directly [McCarty]. All of this results in a differing variety of intermediate and end products depending upon the species of microorganisms present, environmental conditions, and amount of time that has transpired.

Petroleum hydrocarbon compounds have been found to fall into categories of bioremediation potential according to their physical classifications.

Ronald M. Atlas [1988] makes the following generalizations about the relative susceptibilities of hydrocarbons to microbial attack:

"(a) When hydrocarbons become available to a microbial community in a complex mixture such as petroleum, biodegradation of most of the hydrocarbon compounds occurs simultaneously but at widely differing rates; the biodegradation of petroleum hydrocarbons varies greatly, leading to sequential disappearance of individual components and to successional changes in the degrading microbial community.

(b) The presence of one hydrocarbon substrate may influence the biodegradation of another in either a positive manner (by co-metabolism) or a negative manner (by toxicity or sparing).

(c) Utilization of C1 - C4 alkanes is restricted to a few specialized species; alkanes in the C5 - C9 range are toxic to many microorganisms but can be biodegraded by some. n-Alkanes of the C10-C22 range usually are readily metabolized. Higher-molecular-weight alkanes tend to be solid waxes and are not readily biodegraded; however, slow biodegradation of n-alkanes up to C44 has been shown.

(d) Iso-alkanes are less readily utilized as compared to n-alkanes; methyl branching can hinder of completely block biodegradation.

(e) Olefins tend to be more toxic and, at least under aerobic conditions, are less readily utilizable than the corresponding alkanes.

(f) Monoaromatic hydrocarbons can be toxic, but in low concentrations many microorganisms rapidly utilize these compounds. Condensed polyaromatics having two to four rings are biodegradable at rates that decrease with the level of condensation. Condensed Polyaromatics with

five or more rings are poor substrates for microbial attack.

(g) Low-molecular-weight cycloalkanes only rarely serve as growth substrates. At low concentrations, cycloalkanes are degraded at moderate rates; highly condensed cycloalkanes are refractory.

(h) Heterocyclic NSO (nitrogen, sulfur, and oxygen) compounds, when not highly condensed, may be subject to limited microbial metabolism. Highly condensed asphaltenes are very resistant to biodegradation."

Promotion of oil degradation results in intermediate compounds of increased mobility [Bossert and Bartha]. One study showed that the amount of water soluble organics in the leachate doubled due to microbial activity on the oil [Dibble and Bartha].

Another major aspect of microbial activity acting upon petroleum hydrocarbons is the production of biosurfactants. Microorganisms, in response to the presence of hydrophobic compounds, will produce enzymes that are surfactants that act to emulsify the hydrocarbon. Such compounds are readily producible by the microorganisms when grown on hydrocarbons [Westlake]. Cooper and Goldenberg found two Bacillus species which produced significant amounts of surfactants and emulsifiers during growth on water-soluble substrates such as sucrose. Generally the production and excretion of biosurfactants is stimulated by the presence of hydrophobic compounds. Contaminants within the porous matrix of the subsurface go into microemulsions and micelles and are dispersed into the soil water. Transfer to the cell where the hydrocarbon can be utilized is readily performed [Wilson and Brown].

Biosurfactants have been found to be anionic or nonionic in nature; no cationic biosurfactants have been found to be produced. Sometimes the structure of the surfactants is determined by the carbon substrate and the

conditions during growth. Some compounds have been found to reduce the surface tension to 25 mN/m and the interfacial tension to less than 1 mN/m [Lang and Wagner]. Zajic et al. [1974] isolated three psuedomonads which readily emulsified no. 6 fuel oil. A paraffin fraction of the oil was utilized to synthesize a high-molecular-weight polysaccharide that acted as an emulsifier.

Biosurfactants are important for hydrocarbon metabolism in that they allow the hydrocarbon utilizer to emulsify and adhere to the oil/water interface. Surfactants produced by microorganisms tend to be lipid structures. The major varieties of lipids found in the cells are fatty acids, glycolipids, lipopolysaccharides, lipoteichoic acids, phospholipids, lipopeptides and neutral lipids. Fatty acids are the main constituents. The structure and length will vary from species to species and according to physiological and environmental conditions. The extracellular lipids excreted by microorganisms have been found to be excellent surfactants Zajic and Mahomedy].

Cooper and Zajic state that "biosurfactants are important because they present a much broader range of surfactant types and properties than the available synthetic surfactants. Furthermore, they are usually biodegradable which reduces the potential of pollution". Currently, research is underway exploring the use of biosurfactants in place of synthetic surfactants in enhanced oil recovery. Regarding bioremediation, laboratory research needs to be conducted to determine if, in some cases at least, the introduction of synthetic surfactants would significantly increase the rate of bioremediation enough to justify the expense of its use in a field setting.

CONCLUSIONS

The following conclusions can be made from the literature searched for this report.

1) A wide range of surfactants are available that are biodegradable under aerobic conditions.

2) Soil, petroleum hydrocarbon and surfactant interactions dictate that for a given set of conditions, only certain surfactants will significantly lower the surface and interfacial tensions and thus be effective.

3) Emulsifiers have been found to increase the rate of bioremediation in certain cases but no research has shown there to be an increase in the extent to which a petroleum product will be degraded.

4) Most petroleum products are a complex mixture of components. Individual petroleum constituents display a range of biodegradation rates based on their classification and physical properties.

5) Laboratory and field testing is needed to quantify the effects of surfactants upon bioremediation rates and extent.

REFERENCES

Anonymous, "Toxic Substances Fact Sheet: Underground Storage Tanks", Dept. of Social and Health Sciences, State of Washington, Nov. 1988.

S.C. Hern, S.M. Melanson, Vadose Zone Modeling of Organic Pollutants, Lewis Publishers, Inc., 1986.

E. Nyer, G. Sklandany, "Relating the Physical and Chemical Properties of Petroleum Hydrocarbons to Soil and Aquifer Restoration", Groundwater Monitoring Review, Vol IX, No. 1, Winter 1989.

J. T. Wilson, C. H. Ward, "Opportunities for Bioreclamation of Aquifers Contaminated with Petroleum Hydrocarbons", from Developments in Industrial Microbiology, Vol. 27, 1989 pp 109-116.

S. B. Wilson, R. A. Brown, " In Situ Bioreclamation; A Cost Effective Technology to Remediate Subsurface Organic Contamination", from Groundwater Monitoring Review, Vol IX, No. 1, Winter 1989, pp 54-60.

J. T. Wilson, R.C. Sims, "In Situ Treatment Design - Surface and Subsurface", from Bioremediation Continuing Education Course, Air and Waste Management Association, Feb. 1989, pp 5-2 to 5-59.

C.H. Ward, M.D. Lee, " In Situ Technologies", from Groundwater Pollution Control, ed. by L. W. Canter, R.C. Knox, Lewis Publishers, 1985.

M.D. Lee, J.M. Thomas, R.C. Borden, P.B. Bedient, J.T. Wilson, C.H. Ward, "Biorestoration of Aquifers Contaminated with Organic Compounds", CRC Critical Reviews in Environmental Control, Vol. 18, Issue 1, 1988.

S. Fogel, M. Findlay, "Bioremediation Techniques for In Situ Treatment of Soil and Water", Cambridge Analytical Associates, Sturbridge, MA, April 1988.

R. D. Swisher, Surfactant Biodegradation, Marcel Dekker, Inc., 1970.

R.D. Swisher, Surfactant Biodegradation, Second Edition, Revised and Expanded, Marcel Dekker, Inc. 1987.

Texas Research Institute, Inc., "Underground Movement of Gasoline on Groundwater and Enhanced Recovery by Surfactants", American Petroleum Institute Publication No. 4317, Sept. 1979.

Texas Research Institute, Inc., "Test Results of Surfactant Enhanced Gasoline Recovery in a Large-Scale Model Aquifer" American Petroleum Institute Publication no. 4390, April 1985

J.H. Nash, "Field Studies of In Situ Soil Washing", EPA report EPA/600/2-87/110 Dec. 1987

G.J. Mulkins-Phillips, J.E. Stewart, "Effect of Four Dispersants on Biodegradation and Growth of Bacteria on Crude Oil", Applied Microbiology, Vol. 28, No. 4, Oct 1974.

R.R. Colwell, J.D. Walker, "Ecological Aspects of Microbial Degradation of Petroleum in the Marine Environment", from CRC Critical Reviews in Microbiology, Vol. 5, Issue 4, Sept 1977.

M.D. Lee, J.T. Wilson, C.H. Ward, "In Situ Restoration Techniques For Aquifers Contaminated With Hazardous Wastes"
Revised 3/31/86.

J.C. Morgan, R.S. Schecter, W.H. Wade, "Recent Advances in the Study of Low Interfacial Tensions", from Improved Oil Recovery by Surfactant and Polymer Flooding, ed. by D.O. Shah, R.S. Schecter, Academic Press Inc., 1977.

W.A. Jury, "Adsorption of Organic Chemicals onto Soil", from Vadose Zone Modeling, ed. by S.C. Hern, S.M. Melanson, Lewis Publishers, 1986, pp 177-189.

D.M. Tuck, P.R. Jaffe, D.A. Crerar, "Enhancing Recovery of Immobile Residual Non-Wetting Hydrocarbons from the Unsaturated Zone Using Surfactant Solutions", from NWWA Petroleum Hydrocarbons Conference, Houston, TX, Nov. 9-11, 1988.

T.J. Robichaux, H.N. Myrick, " Chemical Enhancement of Biodegradation of Crude Oil Pollutants", from Journal of Petroleum Technology, Vol 16, Jan. 1972.

T.D. Brock, D.W. Smith, M.T. Madigan, Biology of Microorganisms, 4th edition, Prentice-Hall. 1984.

P.L. McCarty, "Bioengineering Issues Related to the In Situ Remediation of Contaminated Soils and Groundwater", from Environmental Biotechnology: Reducing Risks from Environmental Chemicals through Biotechnology, edited by G.S. Omenn, Plenum Press. 1988.

I. Bossert, R. Bartha, "The Fate of Petroleum in Soil Ecosystems", from Petroleum Microbiology, ed. by R.M. Atlas, Macmillan Publishing, pages 435 - 474. 1984.

J.T. Dibble, R. Bartha, "Leaching aspects of Oil Sludge Biodegradation in Soil, from Soil Science, Vol. 127, pages 365-370

D.W.S. Westlake, "Heavy Crude Oil and Oil-Shales: Tertiary Recovery of Petroleum from Oil-Bearing Formations", from Petroleum Microbiology, ed. by R. M. Atlas, Macmillan Publishing, pages 537 - 552. 1984.

D. Cooper, B. Goldenberg, "Surface Active Agents from Two Bacillus Species", from Applied and Environmental Engineering, V. 53, No. 2, pages 224 - 229. Feb. 1987.

S. Lang, F. Wagner, "Structures and Properties of Biosurfactants", from Biosurfactants and Biotechnology, ed. by N. Kosaric, W.L. Carins, N. Gray. Marcel Dekker, Inc., pages 21 - 45. 1987.

J.E. Zajic, B. Supplison, B. Volesky, "Bacterial Degradation and Emulsification of No. 6 Fuel Oil", from Environmental Science Technology, V. 8. pages 664 - 668. 1974.

J.E. Zajic, A.Y. Mahomedy, "Biosurfactants: Intermediates in the Biosynthesis of Amphiphatic Molecules in Microbes", from Petroleum Microbiology, ed. by R.M. Atlas, Macmillan Publishing, pages 221 - 297. 1984.

D.G. Cooper, J.E. Zajic, "Surface Active Compounds from Microorganisms", from Advances in Applied Microbiology, V 26, pages 229 - 253. 1980.